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NOTES ON GEOGRAPHIC DISTRIBUTION

First confirmed records with molecular data of the terrestrial flatworm *Bipalium kewense* Moseley, 1878 (Tricladida, Geoplanidae) in Madagascar, Egypt, and South Africa

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Abstract. The land flatworm *Bipalium kewense* Moseley, 1878 is the most widely distributed invasive species of land flatworm, being found in over 78 countries. A specimen was recently collected in an urbanized part of the capital city of Antananarivo in Madagascar, identified on the basis of external morphology and later confirmed by molecular methods. This represents the first strictly confirmed occurrence of the species in Madagascar. Previous African records of *Bipalium kewense* are summarized, and two records from continental Africa (Egypt and South Africa) are confirmed by molecular barcoding.

Key words. Africa, Bipaliinae, Continenticola, Indian Ocean, mitochondrial genome, Platyhelminthes

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INTRODUCTION

In recent years, there has been particular interest in invasive terrestrial flatworms, or Geoplanidae, as many Asian, South American, or Oceanian species have increasingly appeared in Europe, North America, or various other regions. These animals have probably been inadvertently introduced via the plant trade (Alvarez-Presas et al. 2014; Carbayo et al. 2016; Justine et al. 2018, 2022, 2024, Mateos et al. 2020; Sluys 2016).

With their hammerhead shape, members of the subfamily Bipaliinae are possibly the most representative and evocative of geoplanids. Of these, *Bipalium kewense* Moseley, 1878, is the most widely distributed introduced species and is now found in over 78 countries worldwide (European Commission 2022), with new observations regularly published (for recent examples, see Cilia 2024; de Luna et al. 2022; de Luna and Boll 2023; Mori et al. 2024; Nicolosi et al. 2025). The colonizing success of this species has been attributed to its ability to reproduce asexually (Hyman 1943). This species is native to the uplands of Indochina (de Beauchamp 1939; Winsor 1983; European Commission 2022), as defined as a peninsula in Southeast Asia between the Bay of Bengal and the South China Sea that comprises Vietnam, Cambodia, Laos, Thailand, western Malaysia, and Myanmar.

In the Afrotropics, *B. kewense* has been sparsely recorded, with the highest concentration of observation occurring in South Africa. The species has been recorded in Cameroon (iNaturalist https://inaturalist.ala.org.au/observations/135218705, accessed 10.04.2025); Cape Verde Islands (Luther 1956); Egypt (Ali 2008; Mori et al. 2023); Kenya (Ogren et al. 1992); Madagascar (Winsor 1983 and this paper); Mauritius (Geba 1909; Winsor 1983),

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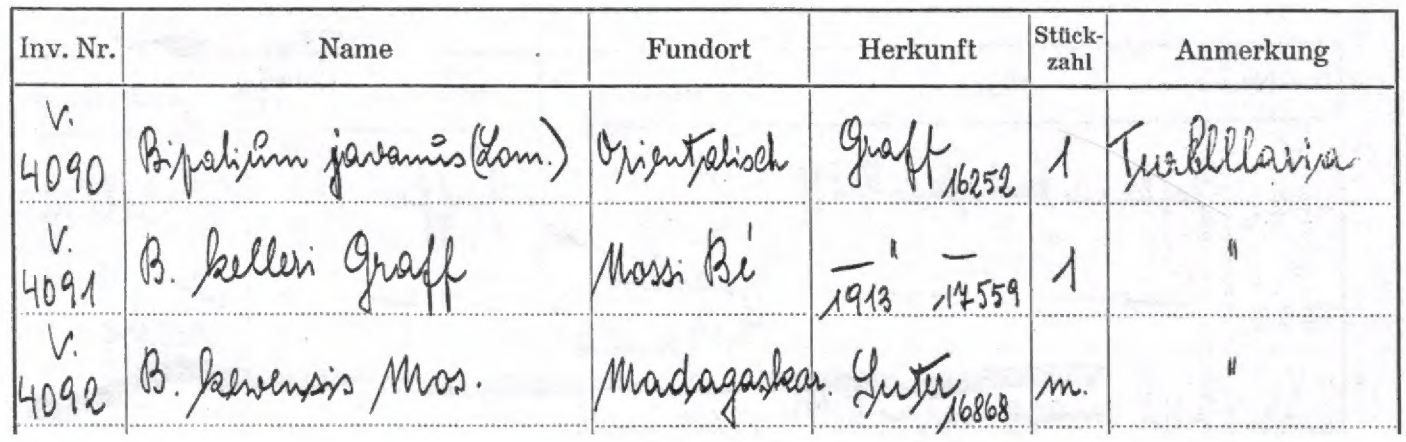

Inv. Nr.	Name	Fundort	Herkunft	Stück-zahl	Anmerkung
V. 4090	Bipalium javanus (Com.)	Orientalisch	Graff 16252	1	Trockillaria
V. 4091	B. kelleri Graff	Mossi Bi	— 1913 — 17559	1	"
V. 4092	B. kewensis Mos.	Madagaskar.	Suter 16868	m.	"

Figure 1. Naturhistorisches Museum (NHM) Wien. Catalogue of the Graff Collection that was donated by the University of Graz in 1974. Entry for V 4092, *Bipalium kewensis*. The "m" in column 5, Stück zahl (number of pieces) stands for "mehrere" (several).

Reunion (de Beauchamp 1948); Rwanda (iNaturalist https://inaturalist.ala.org.au/observations/26957711, accessed 10.04.2025); Saint Helena (Ball, 1977); São Tomé and Príncipe (Sluys et al. 2017); South Africa (Trimen 1887; Fisk 1889; Jameson 1907), and Zimbabwe (Winsor 1983).

In his comprehensive review of *B. kewense*, Winsor (1983) noted that the specimen collection V4092 "*Bipalium kewensis*" from the Naturhistorisches Museum Wien (NHM Wien) (Vienna) was from "Madagascar" (Figure 1), and this information has been cited in later publications (e.g. Mori et al. 2024). The collection V4092, comprising 13 non-sexual typical specimens of *B. kewense* (LW unpublished data) was originally donated or more likely sold by Henry Suter to the Zoological Institute of the University of Graz, Austria. Henry Suter (1841–1918), a Swiss national, was a talented amateur naturalist who had emigrated to New Zealand. He supplemented his meagre income by selling natural history specimens to overseas collectors and museums. Friends and colleagues gave him duplicate specimens from their collections that he was later able to sell, and as Suter had never been to Madagascar (Hyde 2017), this was possibly the source of specimen collection V4092.

The specimen collection V4092, originally from the Zoological Institute of the University of Graz, was transferred to the NHM around 1974 (R. Schuster to L. Winsor, letter dated 9 February 1976). Interestingly, there is no mention of this specimen record in Madagascar in Graff (1899), nor was it mentioned by Mell (1903) or Geba (1909), both from the Graz school, nor by de Beauchamp (1948) in their studies and review of terrestrial flatworms from Madagascar. In fact, none of these authors reported the presence of *B. kewense* in Madagascar, although Geba (1909) described *Placocephalus isabellinus* from Mauritius, later synonymized with *B. kewense* (Winsor 1983), and specimens of *B. kewense* had been found on La Reunion by M. Remy (de Beauchamp 1948). Details of the exact provenance and date of Suter's specimens comprising V4092 are unknown. Therefore, although strongly suspected, the presence of *B. kewense* in Madagascar has yet to be confirmed.

A specimen of Bipaliinae was recently found in Antananarivo, the capital city of Madagascar (Figure 2). It was subsequently identified from a photograph of the live specimen as *B. kewense* (Figure 3). The speci-

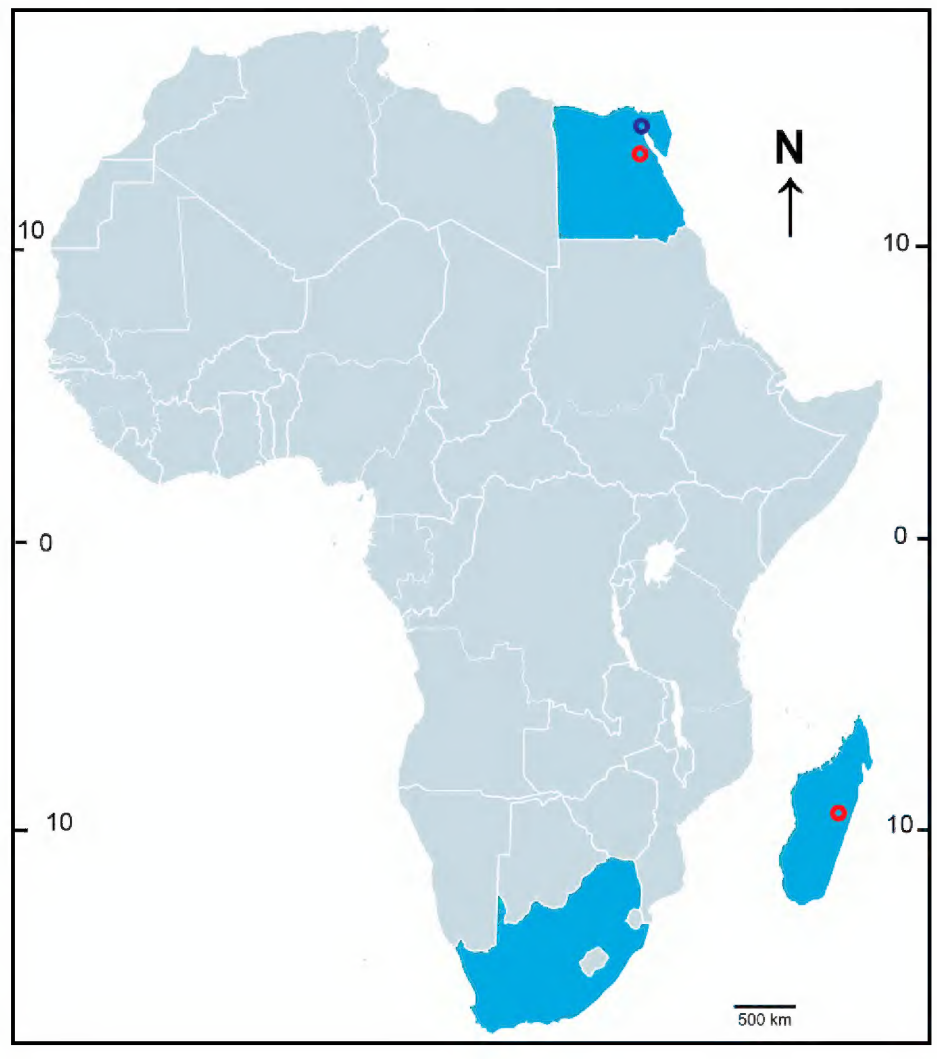

Figure 2. Map of the sampling places. Blue: countries in which *Bipalium kewense* has been reported besides the records from this study. Blue dot: Cairo in Egypt where the species has been reported. Red dots: new geographical records with molecular information. There is no red dot indicated for South Africa since the provenance of the specimen from that county is unknown.

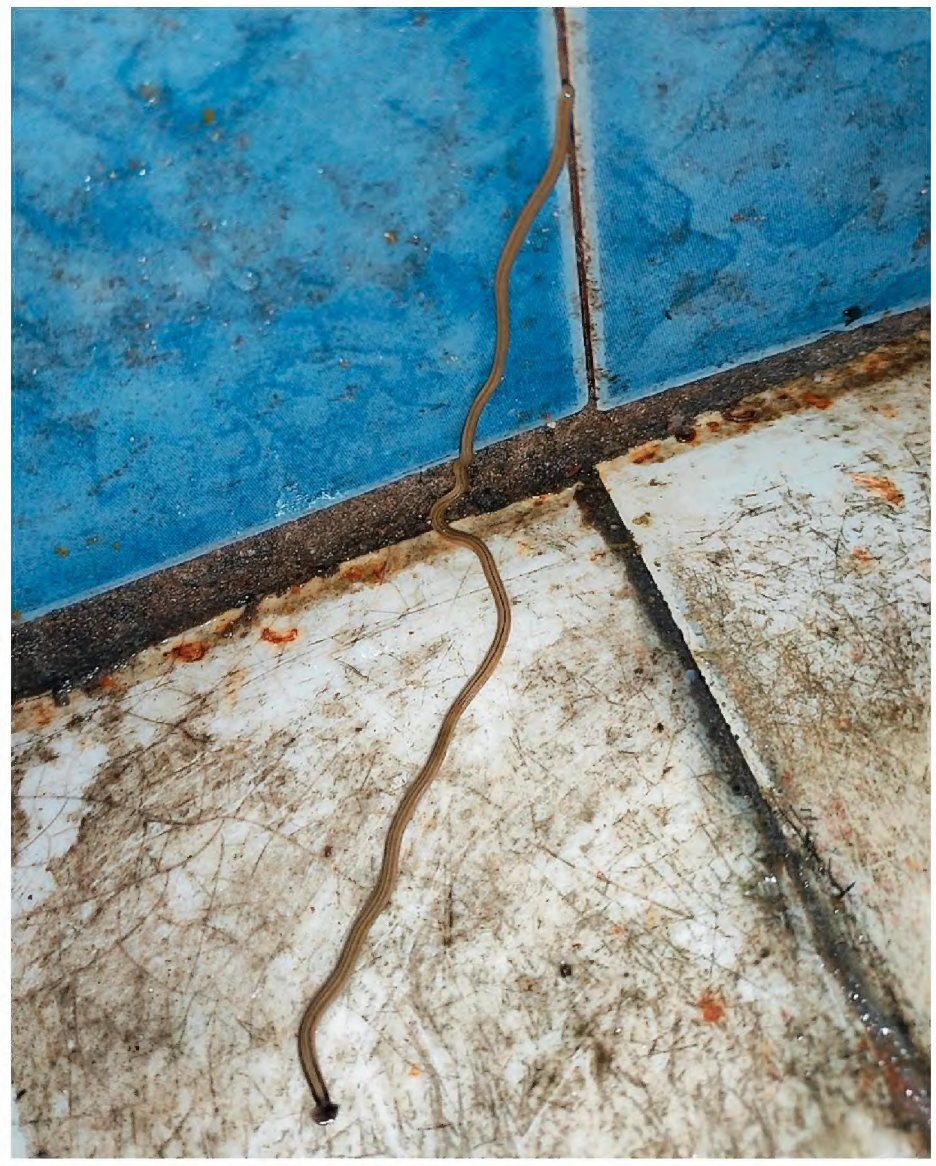

Figure 3. *Bipalium kewense*, living specimen (MNHN JL518) photographed in Antananarivo, Madagascar.

men was deposited in the Muséum National d'Histoire Naturelle (MNHN) in Paris and its identification was corroborated by sequencing its mitochondrial genome and comparing it with the reference sequence. Unconfirmed observations from iNaturalist are also discussed.

To extend our knowledge on the presence of *B. kewense* in continental Africa, we provide two records from Egypt and South Africa, which are the first records confirmed by molecular barcoding (partial cox1 gene).

METHODS

On 27 March 2023 a specimen of *B. kewense* was found in a highly urbanized environment, in the kitchen of a house in the '67Ha' residential area, North-Ouest part of the capital city of Antananarivo, Analamanga Region). The specimen was transferred out of the country with permit 151/23/MEDD/SG/DGGE/DAPRNE/SCBE.Re and forwarded to the Muséum National d'Histoire Naturelle (MNHN) in Paris, France where it was identified by two of us (LW and JLJ) and registered in the collection under accession number MNHN JL518. The specimen from Egypt was collected by one of us (RRR) in August 2016 in an experimental farm on the campus of Assiut University (27°11'07.1"N, 031°09'29.0"E) and owned by the university. The specimen was fixed in ethanol and sent to the MNHN to be registered in the collections as MNHN JL303. The last specimen was collected on 11 August 2019 by the French plant inspection services (Direction Régionale de l'Alimentation, de l'Agriculture et de la Forêt, DRAAF, Île de France) at Paris Charles de Gaulle Airport (Roissy-en-France, France). It was found in a shipment of unidentified plants of the genus *Agapanthus* L'Her. originating from South Africa (exact location unknown) ; this specimen was sent to one of us (JLJ) for identification and deposition in the MNHN collection (accession number MNHN JL350).

For next-generation sequencing of the specimen MNHN JL518 from Madagascar, a 1 cm portion was cut and sent to the Plateforme d'Analyses Génomiques of the Institut de Biologie Intégrative et des Systèmes (IBIS, Université Laval, Québec, Canada). Genomic DNA was extracted, mechanically fragmented using a Covaris M220 instrument (Covaris, Woburn, MA, USA) and a library was prepared using an NEBNext Ultra II kit (New England Biolabs, Ipswich, MA, USA) following the manufacturer's instructions. The library was then sequenced on an Element Bioscience AVITI platform (San Diego, CA, USA). The 150-bp paired-end reads were quality filtered using fastp (Chen et al. 2018), leaving a total of ca. 206 M clean reads that were assembled with SPAdes v. 4.0 and a k-mer of 125 (Bankevich et al. 2012). Once identified, the contig containing the mitochondrial genome was annotated with the help of MITOS (Donath et al. 2019) and the available reference from GenBank (MK455837).

For molecular barcoding of the specimens MNHN JL303 and MNHN JL350, the cox1 gene was amplified by routine PCR and sequenced on a Sanger platform, as described in Justine et al. (2018). All these steps were performed at the MNHN facilities.

RESULTS

Phylum Platyhelminthes Minot, 1876
Subphylum Rhabditophora Ehlers, 1985
Order Tricladida Lang, 1884
Suborder Continenticola Carranza, Littlewood, Clough, Ruiz-Trillo, Baguñà & Riutort, 1998
Superfamily Geoplanoidea Stimpson, 1857
Family Geoplanidae Stimpson, 1857
Subfamily Bipaliinae von Graff, 1896
Genus *Bipalium* Stimpson, 1857

***Bipalium kewense* Moseley, 1878**

Figures 3-6

First report. MADAGASCAR — **Anamalanga** • Antananarivo; 18°54′08.8″S, 047°30′32.1″E; 27.III.2023; Marcel Koken & Suzelyce Eva Soavolamanoro leg.; collected in a 15 mL Falcon tube with 90% ethanol; GenBank: PQ645074; in ethanol, MNHN JL518.

Figure 4. *Bipalium kewense*, specimen MNHN JL518 fragmented into three pieces following successive attempts to collect it.

Figure 5. *Bipalium kewense*, specimen MNHN JL518, remaining anterior part of the body after a first attempt to collect it. This picture was used for identification because of its higher definition.

Figure 6. *Bipalium kewense*, specimen MNHN JL518, fragmented part exhibiting both the dorsal and ventral sides.

Identification. The specimen (Figure 3) initially measured approximately 24.5 cm long and 3.2 mm in maximum width, with an elongate body, slightly rounded dorsally, and tapering slightly towards the extremities. When collecting the specimen, it immediately broke into several smaller pieces (Figure 4). The black-brown semilunate headplate has slightly recurved lappets, and the posterior tip is bluntly rounded on the partial specimen used for identification (Figure 5), which is congruent with its earlier fragmentation. There are five fairly evenly spaced dorsal-dorsolateral longitudinal stripes. A fine black-brown median stripe beginning well behind the headplate thickens over the pharyngeal area and continues to the hind end. A black-brown incomplete transverse band (interrupted collar) lies immediately behind the headplate, from which the paired lateral and marginal stripes arise. The lateral nut-brown stripes have diffuse margins and are separated from the median stripe by about the same width of ivory ground colour, and from the fine paired nut-brown marginal stripes by a similar interval of brown-beige ground colour. In Figure 6 showing the fragmented specimen, the dorsal side (upper right of the photo) has dark median and paired dorsal-dorsolateral stripes, and the ventral surface (lower left of the photo) has a median, narrow, white creeping sole delineated on either side by the violet-grey longitudinal stripes. The specimen exhibited the typical external morphology of *B. kewense* and can be distinguished from similar species by the presence of an incomplete transverse band immediately behind the headplate and the five dark, fairly evenly spaced dorsal-dorsolateral longitudinal stripes that all begin at or just behind the headplate (Winsor 1983).

After assembly of the sequencing reads, it was possible to retrieve a 15,729-bp contig corresponding to the mitogenome (GenBank: PQ645074). Unlike the 15,666-bp reference sequence from GenBank (MK455837, specimen from France), it was not possible to circularize the contig, although it is being displayed as circular for easier reading (Figure 7). The contig broke in an area between genes rrnL and cob, which is now known in other Geoplanidae to harbour repeated sequences (Gastineau et al. 2024). After removal and trimming of the non-coding parts that were not shared with MK455837, only four differences were found between both sequences, out of which two concerned coding regions. One was a silent single nucleotide polymorphism in ND4, the other one an indel in rrnL.

The two specimens from Egypt and South Africa had identical partial cox1 sequences, which were registered in GenBank under accession numbers PV032007 and PV032008 for specimens MNHN JL303 and MNHN JL350, respectively. Megablast queries returned a 100% identity with various cox1 sequences of *B. kewense*, including the two complete aforementioned mitogenomes.

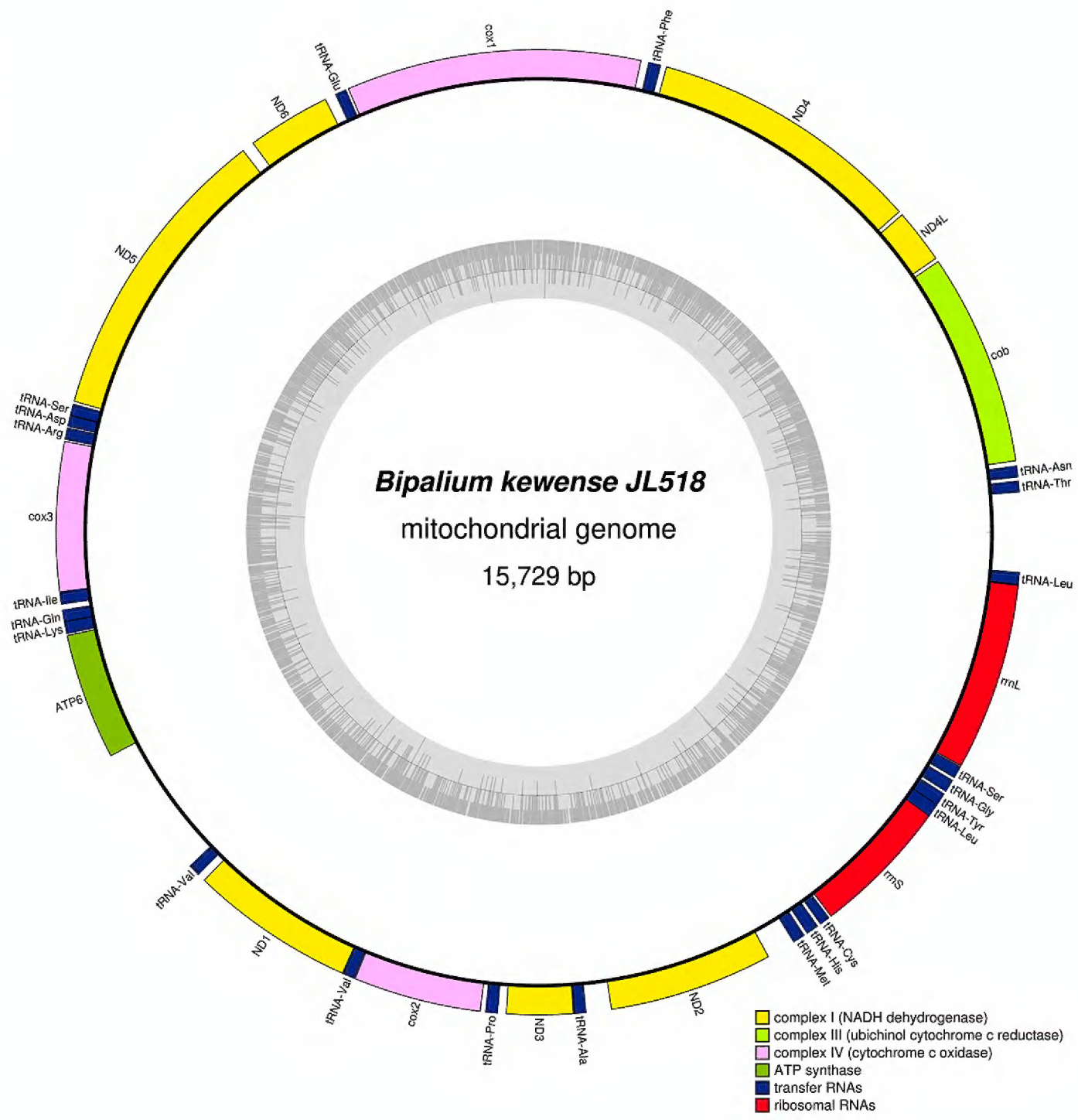

Figure 7. Map of the mitochondrial genome of *Bipalium kewense* MNHN JL518. The mitogenome is represented as circular for easier reading, although it could not be circularized.

DISCUSSION

Madagascar has a rich fauna of Bipaliinae, with 10 species of *Humbertium* Ogren & Sluys, 2001, and 16 species of *Diversibipalium* Kawakatsu, Ogren, Froehlich & Sasaki, 2002 (Solà et al. 2023) described so far. In terms of external morphology, *Humbertium ithorense* Solà et al., 2023 might be confused with *B. kewense*. It has four dark dorsal longitudinal stripes (five dorsal-dorsolateral stripes in *B. kewense*), thickening of the stripes forming a dark patch on either side of the neck that continues to encircle the anterior margin of the headplate (a distinct interrupted transverse band that only extends dorsally and ventrally in *B. kewense*), with yellowish ground colour, and exhibiting on the headplate the pale "exclamation mark" at the beginning of the pale median dorsal stripe seen on many species of *Humbertium* (absent in *B. kewense*). None of the other 25 Madagascan species have a combination of external characters approaching those of *B. kewense*.

The current study represents the first confirmed occurrence of *B. kewense* in Madagascar, but it is noted that iNaturalist (2024a, 2024b) also lists two putative observations, both of which occurred in the last two years south of Antananarivo, at distances of ca. 90 km and ca. 400 km from the capital city. These observations were from either a rural area or Ranomafana National Park, and we emphasize that this species is highly likely to have widespread distribution in the central highlands. Data sharing between scientists and the general public through platforms like iNaturalist for scientists or Facebook groups can greatly assist researchers in efficiently mapping the distribution of invasive species.

The impossibility to circularize the mitogenome of specimen MNHN JL518 interrogates whether our previous sequencing (Gastineau et al. 2019) missed a repeated region, or if these repetitions are distributed unequally among specimens. Such questions might only be solved using long-read sequencing technologies (e.g. Gastineau et al. 2023, 2024), which is beyond the scope of our study. On the other hand, the strong conservation of the mitogenome, even in the non-coding parts, corroborates and strengthens previous assumptions based on the high conservation of the cox1 gene among various specimens of *B. kewense* from Spain, the Azores, Cuba, Monaco, Portugal, Continental France, Guadeloupe, Martinique, and French Guiana (Justine et al. 2018).

Questions remain regarding the "when" and "how" of the introduction of *B. kewense* to Madagascar. As previously stated, the exact provenance is unknown for the *B. kewense* specimens from Madagascar with the NHM number V4092 . However, the species had already been introduced to Madagascar in the 19th or early 20th century because V4092 had been donated before Suter's death in 1917. It would be one of the "old" group of species introduced into European countries and their colonies by horticulturists, the main driver being the use of the "Wardian case", invented in 1842, which facilitated the safe transport and exchange of living plants. However, this unluckily lead to the inadvertent introduction of cryptic exotic animal species, including flatworms, with soil (Winsor et al. 2004). In South America and the Indo-Pacific regions, banana rhizomes have been invoked as likely passive vehicles for the inadvertent transport of land planarians (Froehlich 1955; Sluys 1995). Notably, a large proportion of the human population of Madagascar has Austronesian ancestry, with forefathers sailing from the Indo-Malay region more than 2000 years ago and introducing to the island hundreds of plants, which could have led to the simultaneous introduction of geoplanids. As stated above, there are other species of Bipaliinae observed in Madagascar (Solà et al. 2023) and on the neighbouring island of Mayotte (Justine et al. 2022). Alternatively, *B. kewense* may have been introduced more recently, in the modern era of globalized plant trade. These explanations do not exclude each other, as all could be true if there were repeated introductions over time.

The impact of plant transplantations in the dispersal of geoplanids is exemplified by specimen MNHN JL350, which could have entered the French territory in this way. This also illustrates that an invasion can occur indirectly (South-East Asia to South Africa to France) in a world of globalized exchanges. *Bipalium kewense* is a well-known species that is already well documented, but non-direct invasions would render the identification of other lesser known or even unknown terrestrial flatworms far more difficult. Clearly, this would increase the difficulty of pinpointing their area of origin, if there was any doubt concerning them. Like for Madagascar, there are records of *B. kewense* in South Africa that go back to the end of the 19th century and beginning of the 20th century (Winsor 1983), and the species now seems to be well established there (Jones 2004; Picker and Griffiths 2017). However, our record is the first with molecular confirmation.

Records of *B. kewense* in North Africa are scarce, as could be expected from places with dry climates. The presence of *B. kewense* has been noted only once in Egypt as far as we are aware (Mori et al. 2023). This observation in Egypt was in Cairo, in the north of the country, near the Nile Delta, which is the most fertile part of the country. Specimen MNHN JL303 was found in Assiut, ca. 400 km south of Cairo in Upper Egypt. This suggests that *B. kewense* might already be spreading in Egypt along the Nile. Our record is also the first with molecular confirmation for North Africa.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

All specimens were transferred from their countries of origin with due authorizations and/or permits.

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Author contributions

Conceptualization: RG. Investigation: RG, AR, RRR, MK, SES, CO, BB, DG, LW, JLJ. Methodology: RG, LW, JLJ. Resources: MK. Supervision: JLJ. Visualization: MK. Project administration: MK, JLJ. Writing – original draft: RG. Writing – review and editing: AR, MK, RRR, SES, CO, BB, DG, LW, JLJ.

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Data availability

All data supporting the findings of this study are available in the main text.

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